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Fractionation and Characterization of Oil Palm (*Elaeis guineensis*) as Treated by Supercritical Water

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Abstract

In order to investigate a potential for the efficient utilization of oil palm (*Elaeis guineensis*), supercritical water treatment (380ºC/100MPa/8s) has been applied to fractionate into water-soluble portion and water-insoluble portion composing of methanol-soluble portion and methanol-insoluble residue for various parts of oil palm such as trunk, frond, mesocarp fibers, shell, empty fruit bunch (EFB) and kernel cake. These fractionated products were then characterized analytically, in which the water-soluble and methanol-soluble portions were determined to be mainly composed of carbohydrate-derived products and lignin-derived products, respectively. Besides, the methanol-insoluble residue was mainly composed of lignin more than 84wt%, and the phenolic hydroxyl contents determined by aminolysis method was higher than the untreated oil palm sample. In addition, an alkaline nitrobenzene oxidation analysis indicated that, the methanol-insoluble residue was less in oxidation products. These lines of findings implicate that the water-soluble portion could be utilized for organic acid production, whereas the methanol-soluble portion and its insoluble residue for phenolic chemical production.

Keywords: oil palm; supercritical water treatment; lignin; carbohydrate-derived products; lignin-derived products; phenolic hydroxyl content; alkaline nitrobenzene oxidation

1. Introduction

The increasing global demand of plant oils for cooking, food additive, cosmetics, industrial lubricants and biodiesel, has made the palm oil production increasing at a vast rate. In the production process for palm oil in Malaysia, Indonesia and Thailand, a large amount of oil palm wastes are generated such as empty fruit bunch (EFB), mesocarp fiber, shell and kernel cake by the palm oil industry. Trunk and frond of oil palm are also generated as unused biomass in the plantation site.

Although some amount of these wastes are utilized as mulching material, organic fertilizer and boiler fuel[1], much of these is not efficiently used and wasted. For efficient utilization of the whole parts of oil palm, our research group elucidated the chemical composition of each part of oil palm as mentioned above. It was, consequently, found that all parts contain cellulose, hemicellulose and lignin, although the hemicellulose composition of monosaccharides was different among different parts. However, lignin in these parts except for kernel cake was similar to that of hardwood lignin[2].

On the other hand, supercritical water treatment (>374ºC, >22.1MPa) has been applied to cellulose[3] and wood[4,5] for its chemical conversion. It was, then, reported that, depending on the reaction condition, various compounds such as saccharides, dehydrated and fragmented compounds and organic acids could be obtained. Lignin in wood was also found to be converted to various useful phenolic compounds by supercritical water treatment.

In this study, therefore, we further applied supercritical water treatment to various parts of oil palm for their efficient utilization, and the potential of oil palm as biomass resources was studied for its various parts such as trunk, frond, mesocarp fiber (hereinafter will be referred to as “mesocarp”), shell, EFB and kernel cake.

2. Materials and Methods

2.1 Sample preparation

Various parts of the oil palm such as trunk, frond, mesocarp, shell, EFB and kernel cake were obtained from Malaysia and these samples were first cleaned and air-dried. The air-dried samples were then pulverized using a Wiley mill (Yoshida Seisakusho, Japan) and a Fritsch mill (Fritsch, Germany) to pass through 70-mesh sieves. The samples were then extracted with ethanol/benzene (1:2, v/v) for 8h in a Soxhlet apparatus. Subsequently these samples were oven-dried at 105ºC for 10h prior to use for experiments.

2.2 Supercritical water treatment

The supercritical water biomass conversion system used in this study was associated with a batch-type reaction vessel made of Inconel-625 with a volume of 5mL[4]. Fig. 1 shows the flowchart of supercritical water (T_c=374ºC, P_c=22.1MPa) treatment and the fractionation of supercritically treated products. The extractives-free oven-dried sample (150mg) was placed in the reaction vessel with 4.9mL distilled water of high performance liquid chromatography (HPLC) grade (Nacalai Tesque Inc., Kyoto). The reaction vessel was then quickly heated by immersing it into the molten tin bath preheated at 500ºC which represents the temperature inside the reaction vessel at an average of 380ºC during the 8s treatment. After that, the reaction vessel was immersed into water bath to quench the reaction. During this treatment, the temperature and pressure in the reaction vessel were monitored by a thermocouple and a pressure gauge.

Eventually, the supercritical water-soluble portion and supercritical water-insoluble residue could be separated by filtration. After 12h refrigeration, the obtained

supercritical water-soluble portion was retrieved by filtration to separate water-soluble portion and water-insoluble residue. The obtained water-insoluble residue was then washed with 10mL HPLC grade methanol (Nacalai Tesque Inc., Kyoto) for 10min by sonication. The methanol-insoluble residue was then separated by filtration from methanol-soluble portion as filtrates. Subsequently, the yields of these fractions were determined quantitatively after complete evaporation of methanol from methanol-insoluble residue. The yield of water-soluble portion was then estimated by subtracting the yield of methanol-soluble portion and methanol-insoluble residue from the initial amount of oven-dried sample used (150mg).

2.3 Characterization of the fractionated portions

Characterization of the water-soluble portion was conducted with HPLC, ion chromatography (IC), capillary electrophoresis (CE) and ultraviolet-visible (UV-Vis) spectrophotometer.

HPLC analysis was carried out to quantify the presence of monosaccharides and decomposed products of saccharides using Shimadzu LC-10A under the following conditions: column, Shodex KS801 and KS802, flow-rate, 1mL/min, eluent, HPLC grade distilled water, column temperature; 80ºC. IC analysis was, on the other hand, carried out to quantify the presence of oligosaccharides and monosaccharides using Dionex ICS-3000 under these conditions: column; CarboPac PA1, column temperature; 35ºC, eluent; gradient-programmed mixture of HPLC grade distilled water, 0.2M sodium hydroxide and 1.0M sodium acetate, flow-rate; 1.0mL/min.

Meanwhile, CE analysis was carried out to quantify the presence of low molecular weight organic acids with an Agilent G1600A under the following conditions: column; fused-silica capillary (104cm x 75μm Φ), buffer; cation pH 5.6

(Agilent Co.), voltage; -30kV, temperature; 15ºC, detector; UV_{270nm}, and characterization of the lignin-derived products was conducted with a Shimadzu UV-2400 UV-Vis spectrophotometer at the wavelength of 205nm.

Characterization of the methanol-soluble portion was conducted with Hitachi G7000M-M9000 gas chromatography-mass spectrometry (GC-MS) for qualitative analysis of low molecular weight products under these conditions: column; Varian CP-Sil 8CB (30m x 0.25mm Φ), injector temperature; 250ºC, column temperature; 30ºC (1min), 30ºC → 250ºC (5ºC/min increment), 250ºC (10min), carrier gas; helium, flow-rate; 1.5mL/min, emission current; 20μA.

For characterization of the methanol-insoluble residue, the determination of Klason lignin and acid-soluble lignin was conducted according to Dence[6] and Whiting et al.[7] Ultraviolet (UV) spectra were recorded on a Shimadzu UV-2400 UV-Vis spectrophotometer. The absorptivity used in the acid-soluble lignin determination was $110 Lg^{-1}cm^{-1}$ for all samples at the wavelength of 205nm. The lignin content in the samples was then determined as the sum of Klason lignin and acid-soluble lignin. These results were compared with lignin content of untreated oil palm samples.

The phenolic hydroxyl content was determined by aminolysis method according to the procedure described by Lai[8] to determine the number of phenolic hydroxyl groups over 100 phenylpropane (C_9) units of lignin (PhOH/100 C_9); gas chromatography (Shimadzu GC14B) with flame ionization detector (FID) was used for the phenolic hydroxyl content under the following conditions: column; Shimadzu stainless steel packed with 10wt% polyethyleneglycol 20M on 60/80 mesh Shincarbon A (2m x 3mm Φ), column temperature; 180ºC (isothermal), injection port temperature; 230ºC, detector temperature; 250ºC, carrier gas; helium. The average

weight of C_9 unit of lignin in oil palm samples was estimated by the weight of syringyl-type and guaiacyl-type C_9 units, considering the S/V ratio.

The alkaline nitrobenzene oxidation treatment method employed in this study is according to Katahira and Nakatsubo,[9] with veratraldehyde (Nacalai Tesque Inc., Kyoto) as the internal standard. In order to quantify the nitrobenzene oxidation products, vanillin acetate, syringaldehyde acetate and *p*-hydroxybenzaldehyde acetate were prepared with the respective reagent grade chemicals (Nacalai Tesque Inc., Kyoto). Quantification of nitrobenzene oxidation products was carried out by gas chromatography (Shimadzu GC14B) with FID under the following conditions: column; Shimadzu CBP-5 column (25m x 0.25mmΦ), column temperature; 100ºC (1 min), 100ºC → 270ºC (5ºC/min increment), 270ºC (10min), injection port temperature; 250ºC, detector temperature; 250ºC, carrier gas; helium, flow-rate; 1.5mL/min.

3. Results and Discussion

As in Table 1, supercritical water treatment (380ºC/100MPa/8s) was applied to various parts of oil palm and the obtained yields for fractionated water-soluble portion and water-insoluble residue were investigated. As a result, the water-soluble portion was 67 to 84 wt% in yield, similar to those in Japanese beech as reported elsewhere.[10] However, the yields of the methanol-soluble portion, separated from the water-insoluble portion, were mostly lower than those of the methanol-insoluble residue. This is, in fact, exactly the opposite of wood sample, such as above mentioned Japanese beech. The yield of the methanol-insoluble residue and methanol-soluble portion, are all dependant on the type of lignin linkages. If more condensed-type

linkages are present, more residue will remain. If less condensed-type linkages are present, with more ether linkages lignin, then more methanol-soluble portion will remain.

The methanol-soluble portion is originally soluble in supercritical water, because both hydrophilic and hydrophobic substances can be dissolved with supercritical water. However, as the supercritical state of water returned to the initial ambient condition, the hydrophobic substances become insoluble and are surfaced as an oily substance. Therefore, washing the residue with methanol is to dissolve such oily substances into the methanol. It was studied by GC-MS and will be shown later that the methanol-soluble portion is mainly composed of lignin-derived products which explain its hydrophobic nature. For methanol-insoluble residue, we have studied by Klason lignin determination, and found that the majority was lignin (84-99%). In addition, due to the low nitrobenzene oxidation products, majority in the residue is condensed-type lignin (83-96%).

In the following, characterization of the water-soluble portion and water-insoluble portion, namely, the methanol-soluble portion and methanol-insoluble residue will be made.

3.1 Characterization of water-soluble portion

In our previous work[3], a similar study has been carried out on microcrystalline cellulose under the condition of the rising time of 13s in the molten tin bath at 550ºC followed by the 380ºC/100MPa treatment in the reaction vessel for 2-8s. The treatment condition is little bit different in this study: due to the longer rising time in the molten tin bath (500ºC), approximately 22s, followed by 380ºC/100MPa treatment in the reaction vessel for 8s. Thus, no oligosaccharides or monosaccharides were

detected; only decomposed products of saccharides such as dihydroxyacetone (DA), levoglucosan (LG), furfural (FR), organic acids and so on were detected as in Table 2 apart from lignin-derived products. The yield of unknown products which was determined by subtracting the identified products from the total is also shown. Only fructose (monosaccharide) was detected but not oligosaccharides for 8s treatment time in the microcrystalline cellulose study[3]. Indeed the reason for not detecting these saccharides is caused by different temperature-rising time. In our study, when the temperature rising time is longer, further decomposition of saccharides is believed to occur during this period. It might be, in addition, due to taxonomical differences in raw materials.

When lignocellulose is treated by supercritical water, the decomposition pathway has been proposed,[3] whereby with prolonged treatment time, organic acids are expected to be formed in high yield. Previously, it was reported that for wood samples treated in supercritical water under the short treatment time, 5s, organic acids were produced as a result of carbohydrate decomposition. However, during more prolonged treatment time, 4min, propyl sidechain of phenylpropane unit of lignin was also decomposed to organic acids with the total yield up to 35%.[5] Therefore, it can be clear that supercritical water treatment is efficient in decomposing carbohydrate portion of oil palm and that the water-soluble portion could be utilized for organic acids production, preferably with prolonged treatment time. Organic acids could be then converted into methane by anaerobic fermentation as reported by Yoshida et al[5].

3.2 Characterization of methanol-soluble portion

For the methanol-soluble portion, GC-MS analyses were performed for all the various parts of oil palm. The total-ion chromatograms of the methanol-soluble portion obtained by GC-MS analysis are shown in Fig. 2. Based on the GC-MS analysis, the molecular weight (MW) and the peaks identified from Fig. 2 are shown in Table 3. Identification of the peaks were conducted with the retention times and mass fragmentation patterns compared with those of the authentic compounds. However, peaks 4, 5 and 6 were determined from the mass fragmentation pattern reported by Hosoya et al.,[11] whereas peaks 16, 17, 19, 23 24, and 25 were determined from Ralph and Hatfield.[12] It could then be elucidated that these identified phenolic compounds must be mainly derived from lignin as indicated by G (guaiacyl-type) and S (syringyl-type) lignins. These compounds become insoluble as oily products when the critical state of water returns to the initial ambient state. Therefore, they are hydrophobic in nature. However, they could be fractionated mainly as the methanol-soluble portion.

From the previous study[4], it was found that in supercritical water, ether linkages are preferentially cleaved and smaller fraction of lignin can be washed out with methanol. As a result, the residue becomes rich in condensed linkages.

For various parts of the oil palm, it could be observed that the higher peaks detected were mainly from syringyl-type phenolic compounds. This is due to more syringyl-type lignin contained in oil palm, as reported previously.[2]

As indicated in Table 3, the corresponding peaks can be seen in Fig. 2 which are from syringyl-type as well as guaiacyl-type phenolic compounds similar to the nature of hardwoods. Thus, the main components of the oil palm such as trunk, frond and EFB show similarity with hardwoods rather than softwoods. Besides that, the

methanol-soluble portion shows the potential for many phenolic compounds to be recovered, as treated by supercritical water.

3.3 Characterization of the methanol-insoluble residue

Table 4 shows the contents of Klason lignin and acid-soluble lignin for the methanol-insoluble residues in various parts of the oil palm. From Table 4, it was found that the lignin content as the sum of Klason lignin and acid-soluble lignin for these parts ranged between 84.1% and 99.2%. This suggests that the methanol-insoluble residue is mainly composed of lignin.

For untreated oil palm, it was found that the sum of Klason lignin and acid-soluble lignin ranged between 20.5% and 44.7%, with acid-soluble lignin in a range between 2.2% and 5.5%. However, acid-soluble lignin in the methanol-insoluble residue is in a range between 0.1% and 1.6% due perhaps to their removal during supercritical water treatment.

Table 4 also shows the number of the phenolic hydroxyl groups (PhOH) upon 100 C_9 units of lignin for the methanol-insoluble residues. Just for comparison, results of the untreated oil palm samples are included. From the methanol-insoluble residues, it is apparent that the residues have more phenolic hydroxyl groups than the untreated oil palm. Previously, it was demonstrated with lignin model compounds that the condensed-type linkages, such as 5-5, were stable during supercritical water treatment, whereas the noncondensed-type ether linkages such as *β*-*O*-4 were easily cleaved by supercritical water hydrolysis.[4] After the cleavage of the noncondensed-type linkages, phenolic hydroxyl groups increase. This explains the reason for the higher phenolic hydroxyl content observed in methanol-insoluble residues and it suggests that many noncondensed-type linkages are cleaved and that the residues are rich in condensed-type linkages.

It can be observed that the increase in phenolic hydroxyl groups over 100 C_9 units of lignin after supercritical water treatment are larger in oil palm mesocarp and EFB, compared with other parts of oil palm. This could be due to the fact that the lignin in oil palm mesocarp and EFB is more susceptible to degradation under supercritical water treatment. Furthermore, for EFB, there is a strong possibility that it would be originally rich in noncondensed-type linkages because the total yield of vanillin and syringaldehyde are nearly 40%, as in Fig. 3. However for mesocarp, such products are low, to be 6%. This indicates that mesocarp was not originally rich in noncondensed-type linkages.

As reported previously,[2] the molar ratio of syringyl to guaiacyl moieties (S/V) as measured by that of syringaldehyde and vanillin was 3.5 in trunk, 1.4 in frond, 1.2 in mesocarp, 0.7 in shell and 2.8 in EFB. In contrast, the methanol-insoluble residues show small or no presence of nitrobenzene oxidation products, as shown in Fig. 3. These results were expected because nitrobenzene oxidation products are mainly derived from the degradation of the noncondensed-type lignin and the fact that most of these linkages are already cleaved under supercritical water treatment as mentioned above. It also suggests that methanol-insoluble residues are rich in condensed-type lignin. As comparison[10], S/V of Japanese beech is 2.3 for untreated sample and none of the oxidation products were present for its methanol-insoluble residue.

4. Conclusions

Various parts of the oil palm were fractionated and characterized after supercritical water treatment. The results presented in this study show that water-soluble portion has the potential to be converted into organic acids, whereas methanol-soluble portion shows the potential for many phenolic compounds to be

recovered. Overall, the characteristics of oil palm as one of the monocotyledonous angiosperms show some similarity to those of hardwoods, one of the dicotyledonous angiosperms. Although further experiments are required for the industrial applications to utilize various oil palm parts, this study can clarify that supercritical water technology is applicable to obtain various useful chemicals from oil palm. These finding shows that the great potential of oil palm not only as oil production plant but also as lignocellulosics.

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Table 1. Yields of fractionated water-soluble portion and water-insoluble residue for various parts of the oil palm as treated by supercritical water at 380ºC/100MPa/8s.

Sample	Yield (wt%)		
	Water - soluble	Water - insoluble	
		Methanol - soluble	Methanol - insoluble
Trunk	82.1	7.5	10.4
Frond	83.5	8.3	8.2
Mesocarp	67.1	5.0	27.9
Shell	70.3	11.3	18.4
EFB	75.6	7.6	16.8
Kernel cake	81.0	2.2	16.8

Table 2. Yields of products in the fractionated water-soluble portion.

Sample source	Proportion based upon untreated sample (wt%)									
	DA	LG	MG	FR	AA	GA	LA	LP	Unknown	Total
Trunk	5.4	1.1	0.4	0.5	6.4	4.8	1.7	12.2	49.6	82.1
Frond	7.2	1.1	0.3	0.2	2.7	2.5	1.9	7.7	59.9	83.5
Mesocarp	6.3	0.8	0.2	0.2	5.1	3.8	1.0	8.7	41.0	67.1
Shell	3.9	1.0	0.1	0.5	3.1	1.6	0.4	9.7	50.0	70.3
EFB	5.5	1.1	-	0.6	2.6	2.0	1.0	8.1	54.7	75.6
Kernel cake[1]	4.8	0.4	0.3	0.2	4.2	2.1	1.1	0	61.1	81.0

DA: Dihydroxyacetone; LG: Levoglucosan; MG: Methylglyoxal; FR: Furfural; AA: Acetic acid; GA: Glycolic acid; LA: Lactic acid; LP: Lignin-derived product;
[1]It contains 6.8% polymeric substances but not lignin.

Table 3. Identified products in the methanol-soluble portion by gas chromatography/mass spectrometry analysis.

Peak	MW	Compound	Origin
1	124	Guaiacol	G
2	138	4-Methylguaiacol	G
3	152	4-Ethylguaiacol	G
4	134	2-Methyl-4-vinylphenol	G
5	148	2-Methyl-4-(1-propenyl) phenol	G
6	148	2-Methyl-4-(1-propenyl) phenol isomer	G
7	154	Syringol	S
8	166	4-Propylguaiacol	G
9		unknown	
10	164	Eugenol	G
11		unknown	
12		unknown	
13	164	*cis*-Isoeugenol	G
14	164	*trans*-Isoeugenol	G
15		unknown	
16	162	4-Propynylguaiacol	G
17	162	1-(4-hydroxy-3-methoxyphenyl)allene	G
18	166	Acetoguaiacone	G
19	182	4-Ethylsyringol	S
20	180	Guaiacylacetone	G
21	196	4-Propylsyringol	S
23	194	*cis*-4-Propenylsyringol	S
24	192	4-Propynylsyringol	S
25	194	*trans*-4-Propenylsyringol	S
26	178	*trans*-Coniferylaldehyde	G
27	196	Acetosyringone	S
28		unknown	
29	210	Syringylacetone	S
31		unknown	
32	194	Ferulic acid	G
33		unknown	

G: guaiacyl-type lignin, S: syringyl-type lignin

Table 4. Klason lignin, acid-soluble lignin contents and the number of phenolic hydroxyl groups over 100 C_9 units of lignin (PhOH/100 C_9) determined by aminolysis method for the methanol-insoluble residues in various parts of the oil palm.

Sample	Methanol-insoluble residue (wt%)					Untreated oil palm (wt%)			
	KL[1]	ASL[2]	Lignin content[3]	Yield of lignin upon untreated oil palm	PhOH/ 100 C_9	KL	ASL	Lignin content	PhOH/ 100 C_9
Trunk	98.6	0.6	99.2	10.3	36.5	19.4	5.5	24.9	28.4
Frond	83.2	0.9	84.1	6.9	53.9	19.6	2.2	21.8	26.3
Mesocarp	85.3	0.7	86.0	24.0	81.3	39.3	5.4	44.7	21.8
Shell	84.6	0.1	84.7	15.6	22.1	37.1	2.2	39.3	13.1
EFB	86.1	1.6	87.7	14.7	72.0	16.0	4.5	20.5	12.8

[1]KL: Klason lignin, [2]ASL: acid-soluble lignin, [3]Lignin content=KL+ASL

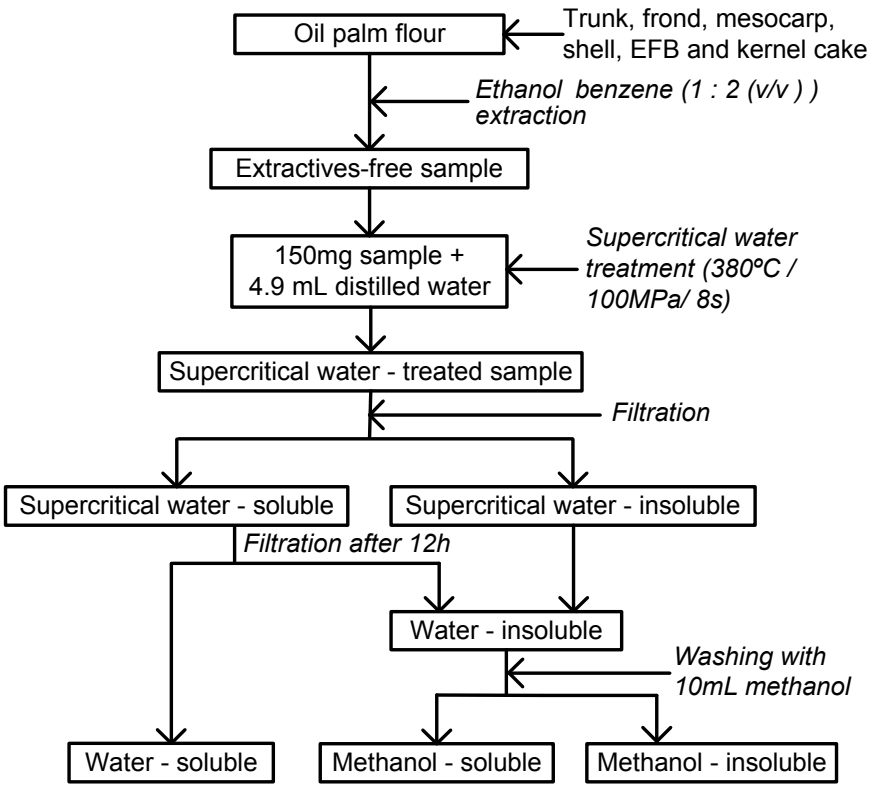

Fig. 1. Fractionation of oil palm treated in supercritical water.

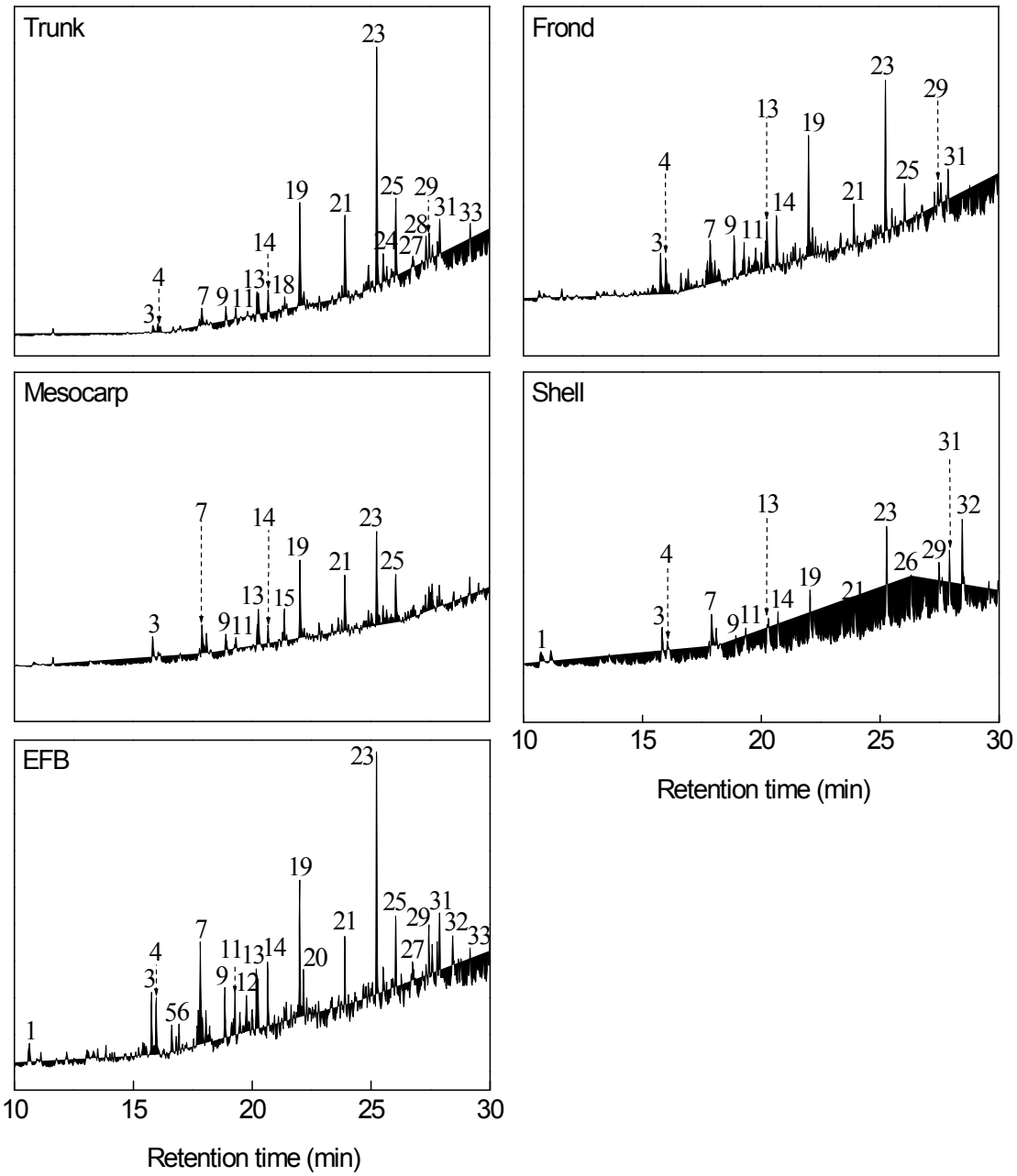

Fig. 2. Total-ion chromatograms for the methanol-soluble portion from various parts of oil palm in gas chromatography/mass spectrometry analyses.

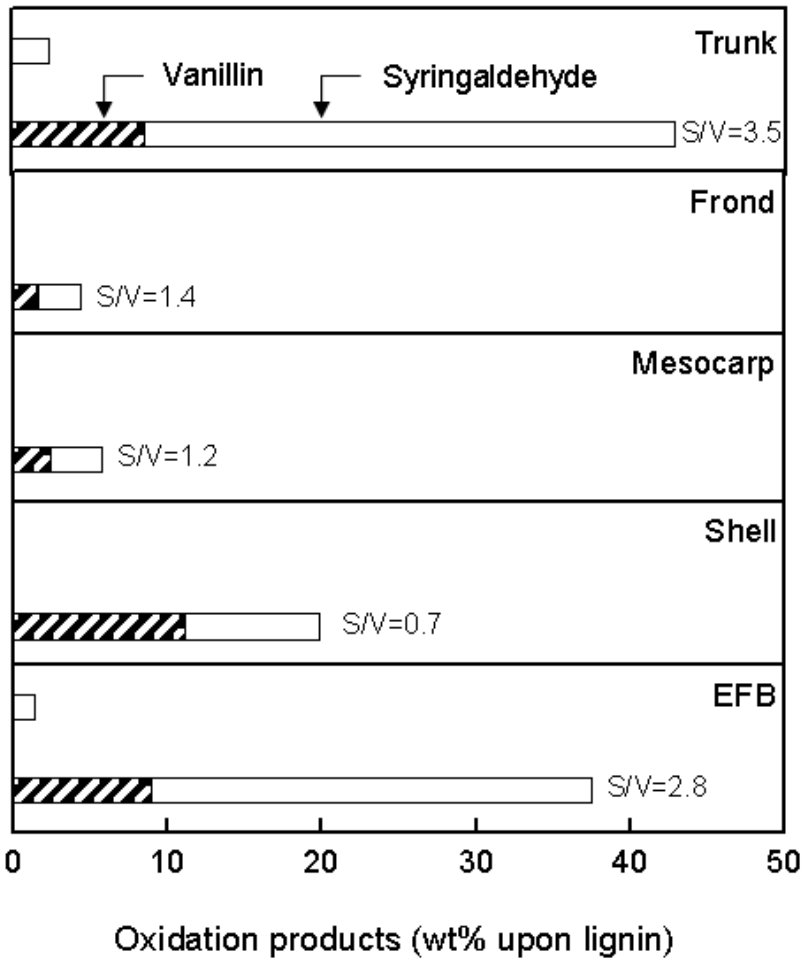

Fig. 3. Yields of the alkaline nitrobenzene oxidation products of the methanol-insoluble residues for various parts of the oil palm (upper) and untreated ~~oil palm~~ ones (lower).